

Amendments to the Specification

Please replace the paragraph beginning on page 6 at line 3, with the following amended paragraph:

“Peptide” or “protein”: According to the present invention, a “peptide” or “protein” comprises a string of at least three amino acids linked together by peptide bonds. The terms “protein” and “peptide” may be used interchangeably. Peptide may refer to an individual peptide or a collection of peptides. Inventive peptides preferably contain only natural amino acids, although non-natural amino acids (*i.e.*, compounds that do not occur in nature but that can be incorporated into a polypeptide chain; ~~see, for example,~~
~~<http://www.cco.caltech.edu/~dadgrp/Unnatstruct.gif>, which displays structures of non-natural amino acids that have been successfully incorporated into functional ion channels~~) and/or amino acid analogs as are known in the art may alternatively be employed. Also, one or more of the amino acids in an inventive peptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, *etc.* In a preferred embodiment, the modifications of the peptide lead to a more stable peptide (*e.g.*, greater half-life *in vivo*). These modifications may include cyclization of the peptide, the incorporation of D-amino acids, *etc.* None of the modifications should substantially interfere with the desired biological activity of the peptide.

Please insert the following paragraph on page 7 after line 20, “**Brief Description of the Drawing**”:

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Please delete the paragraph, beginning at page 9 at line 12, which begins “*Figure 5* shows a Kaplan-Meier”

Please replace the paragraph, beginning on page 9 at line 15, with the following amended paragraph:

Figure 6 5 shows ~~the data obtained from the top 22 genes with *Wnt5a* at the top of the list. The figure also show~~ a diagram of the *Wnt5a* and *Wnt1* signaling pathways.

Please replace the paragraph, beginning on page 9 at line 17, with the following amended paragraph:

Figure 7 6 shows the data from real time PCR analysis of three cell lines, one with low *Wnt5a* expression (which scored as having low expression in the gene chip analysis), one with high *Wnt5a* expression (which scored as having high expression in the gene chip analysis), and one with intermediate *Wnt5a* expression, an originally low scoring cell line which had been transfected with a vector designed to express *Wnt5a*. The parent and transfected cell line were also analyzed for WNT5A protein abundance using Western blot analysis and immunohistochemical staining.

Please replace the paragraph, beginning on page 10 at line 1, with the following amended paragraph:

Figure 8 7 shows the dramatic changes in cell morphology and cytoskeletal organization upon transfection of the parental cell line with a vector driving *Wnt5a* expression. The parental cell line is spindle shaped with few points of attachment to the culture plate and disorganized actin filaments. The transfectants are broader and flatter with many extensions and highly polarized actin filaments.

Please replace the paragraph, beginning on page 10 at line 6, with the following amended paragraph:

Figure 9 ~~8~~ 9 shows the results of experiments done to look at possible cross talk between the *Wnt5a* and *Wnt1* pathways. Beta-catenin was localized to the cytoplasm indicating that the *Wnt1* pathway is not active. The downstream target of *Wnt5a*, protein kinase C, was also observed to be phosphorylated, especially the mu and alpha/beta isoforms, indicating that the expected *Wnt5a* pathway is active.

Please replace the paragraph, beginning on page 10 at line 11, with the following amended paragraph:

Figure 10 ~~9~~ 9 shows scratch assay and Boyden chamber assay results for the parent cell line as well as the transfected cell line. The results from these two standard assays show that increased cell movement and invasiveness correlate with increased *Wnt5a* expression.

Please replace the paragraph, beginning on page 10 at line 14, with the following amended paragraph:

Figure 11 ~~10~~ 10 shows that the transition from low to high *Wnt5a* expression is not associated with increasing amounts of the G protein coupled receptor, frizzled 5 (fzd5). Also shown are results indicating that an antibody to fzd5 can attenuate or reverse the phenotype that increased *Wnt5a* would normally produce.

Please replace the paragraph beginning on page 14 at line 21, with the following amended paragraph:

The sequence of the mRNA of *Homo sapiens* wingless MMTV integration site family, member 5a (*Wnt5a*) is shown below:

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1. attaatctctg gctccacttg ttgctcggcc cagggttgagg agaggacgga gggtagccgc
61 agcgggttcc tgagtgaatt acccaggagg gactgagcac agcaccaact agagaggggt
121 caggggggtgc gggactcgag cgagcaggaa ggaggcagcg cctggcacca gggctttgac
181 tcaacagaat tgagacacgt ttgtaatcgc tggcgtgccc cgcgcacagg atcccagcga
241 aaatcagatt tcctggtgag gttgcgtggg tggattaatt tggaaaaaga aactgcctat
301 atcttgccat caaaaaactc acggaggaga agcgcagtca atcaacagta aacttaagag
361 acccccgatg ctcccctggt ttaacttgta tgcttgaaaa ttatctgaga gggaataaac
421 atcttttcct tcttccctct ccagaagtcc attggaatat taagcccagg agttgctttg
481 gggatggctg gaagtgcaat gtcttccaag ttcttcctag tggctttggc catatttttc
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541	tccttcgccc	aggttgtaat	tgaagccaat	tcttggtggt	cgctaggtat	gaataaccct
601	gttcagatgt	cagaagtata	tattatagga	gcacagcctc	tctgcagcca	actggcagga
661	ctttctcaag	gacagaagaa	actgtgccac	ttgtatcagg	accacatgca	gtacatcgga
721	gaaggcgcg	agacaggcat	caaagaatgc	cagtatcaat	tccgacatcg	acggtggaac
781	tgcagcactg	tggataaac	ctctgttttt	ggcagggtga	tgcagatagg	cagccgcgag
841	acggccttca	catacgccgt	gagcgcagca	gggggtggtga	acgccatgag	ccgggcgtgc
901	cgcgaggcg	agctgtccac	ctgcggctgc	agccgcgccc	cgcgccccaa	ggacctgccg
961	cgggactggc	tctggggcgg	ctgcggcgac	aacatcgact	atggctaccg	ctttgccaa
1021	gagttcgtgg	acgcccgcga	gcgggagcgc	atccacgccca	agggctccta	cgagagtgt
1081	cgcacctcca	tgaacctgca	caacaacgag	gccggccgca	ggacggtgta	caacctggct
1141	gatgtggcct	gcaagtgcc	tgggggtgtcc	ggctcatgta	gcctgaagac	atgtctggctg
1201	cagctggcag	acttccgcaa	ggtgggtgat	gccctgaagg	agaagtacga	cagcgcggcg
1261	gccatgcggc	tcaacagccg	gggcaagttg	gtacagggtca	acagccgctt	caactcgcgc
1321	accacacaag	acctggtcta	catcgacccc	agccctgact	actgcgtgcg	caatgagagc
1381	accggctcgc	tgggcacgca	gggccgcctg	tgcaacaaga	cgtcggaggg	catggtggc
1441	tgcgagctca	tgtgctgcgg	ccgtgggtac	gaccagttca	agaccgtgca	gacggagcgc
1501	tgccactgca	agttccactg	gtgctgtctac	gtcaagtgca	agaagtgcac	ggagatcgtg
1561	gaccagtttg	tgtgcaagta	gtgggtgcca	cccagcactc	agccccgctc	ccaggacccg
1621	cttattttata	gaaagtacag	tgattctgggt	ttttggtttt	tagaaatatt	ttttattttt
1681	ccccagaat	tgcaaccgga	accatttttt	ttcctgttac	catctaagaa	ctctgtgggt
1741	tattattaat	attataatta	ttatttggca	ataatggggg	tgggaaccac	gaaaaatatt
1801	tattttgtgg	atctttgaaa	aggtaataca	agacttcttt	tggatagtat	agaatgaagg
1861	gggaaataac	acatacccta	acttagctgt	gtgggacatg	gtacacatcc	agaaggtaaa
1921	gaaatacatt	ttctttttct	caaatatgcc	atcatatggg	atgggtaggt	tccagttgaa
1981	agagggtggt	agaaatctat	tcacaattca	gcttctatga	ccaaaatgag	ttgtaaattc
2041	tctggtgcaa	gataaaaggt	cttgggaaaa	caaaacaaaa	caaaacaaac	ctcccttccc
2101	cagcagggct	gctagcttgc	tttctgcatt	ttcaaaatga	taatttacia	tggaggaca
2161	agaatgtcat	atttctcaagg	aaaaaaggta	tatcacatgt	ctcattctcc	tcaaatattc
2221	catttgcaga	cagaccgtca	tattctaata	gctcatgaaa	tttgggcagc	agggaggaaa
2281	gtccccagaa	attaaaaaat	ttaaaaactc	tatgtcaaga	tgttgatttg	aagctgttat
2341	aagaattggg	attccagatt	tgtaaaaaaga	cccccaatga	ttctggacac	tagatttttt
2401	gtttggggag	gttggcttga	acataaatga	aatatcctgt	attttcttag	ggatacttgg
2461	ttagtaaatt	ataatagtag	aaataataca	tgaatcccat	tcacaggttt	ctcagcccaa
2521	gcaacaagg	aattgcgtgc	cattcagcac	tgcaccagag	cagacaacct	atttgaggaa
2581	aaacagtga	atccaccttc	ctcttcacac	tgagccctct	ctgattcctc	cgtgttgtag
2641	tgtgatctg	gccacgtttc	caaacggcag	ctccactggg	tcccctttgg	ttgtaggaca
2701	ggaaatgaaa	cattaggagc	tctgcttggga	aaacagttca	ctacttaggg	atttttggtt
2761	cctaaaaact	ttattttgag	gagcagtagt	tttctatgtt	ttaatgacag	aacttggcta
2821	atggaattca	cagagggtgt	gcagcgtatc	actgttatga	tctgtgtttt	agattatcca
2881	ctcatgcttc	tcctattgta	ctgcagggtg	accttaaaac	tgttcccagt	gtacttgaac
2941	agttgcattt	ataagggggg	aaatgtgggt	taatggtgcc	tgatatctca	aagtcttttg
3001	tacataacat	atatatatat	atacatatat	ataaatataa	atataaatat	atctcattgc
3061	agccagtgat	ttagattttac	agcttactct	ggggttatct	ctctgtctag	agcattgttg
3121	tccttccactg	cagtccagtt	gggattattc	caaaagtttt	ttgagtcttg	agcttgggct
3181	gtggccccgc	tgtgatcata	ccctgagcac	gacgaagcaa	cctcgtttct	gaggaagaag
3241	cttgagttct	gactcactga	aatgcgtgtt	gggttgaaga	tatctttttt	tcttttctgc
3301	ctcaccctt	tgtctccaac	ctccatttct	gttcactttg	tggagagggc	attacttggt
3361	cgttatagac	atggacgtta	agagatattc	aaaactcaga	agcatcagca	atgtttctct
3421	tttcttagtt	cattctgcag	aatggaaacc	catgcctatt	agaaatgaca	gtacttatta
3481	attgagtccc	taaggaatat	tcagcccact	acatagatag	cttttttttt	tttttttttt
3541	ttttaataag	gacacctctt	tccaaacagg	ccatcaaata	tgttcttatc	tcagacttac
3601	gttgttttta	aagtttgga	agatacacat	cttttcatac	cccccttag	gaggttgggc
3661	tttcatatca	cctcagccaa	ctgtggctct	taatttattg	cataatgata	tccacatcag
3721	ccaactgtgg	ctctttaatt	tattgcataa	tgatattcac	atccccctcag	ttgcagtga

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3781 ttgtgagcaa aagatcttga aagcaaaaag cactaattag tttaaaatgt cacttttttg
3841 gtttttatta tacaaaaacc atgaagtact ttttttattt gctaaatcag attgttcctt
3901 tttagtgact catgtttatg aagagagttg agtttaacaa tcctagcttt taaaagaaac
3961 tattttaatgt aaaatattct acatgtcatt cagatattat gtatatcttc tagcctttat
4021 tctgtacttt taatgtacat atttctgtct tgcgtgattt gtatatttca ctggttttaa
4081 aaacaaacat cgaaaggctt attccaaatg gaag (Seq. ID No.: 2)

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Please replace the paragraph, beginning on page 20 at line 11, with the following amended paragraph:

In other embodiments, agents may be screened for their ability to inhibit or knock out the *Wnt5a* pathway as shown in Figure 6 5. In one embodiment, agents may be screened for their ability to block the binding of WNT5A to its receptor, frizzled 5. An agent able to block this binding interaction could possibly attenuate or reverse the phenotypes that increased WNT5A would normally produce, such as increased cell movement an invasiveness.

Please replace the paragraph beginning on page 30 at line 22, with the following amended paragraph:

Cultured cells were collected and mRNA isolated as described (Khan *et al.* "DNA Microarray technology: the anticipated impact on the study of human disease" *Biochim. Biophys. Acta* 1423:17-28, 1999; ~~www.nhgri.nih.gov/DIR/microarray~~; each of which is incorporated herein by reference). Samples underwent a series of controls for quality of mRNA, labeling and hybridization, as well as sample integrity (including genotyping DNA from all samples with five dinucleotide markers from four different chromosomes to insure individuality). The entire coding sequence of the p16 gene and exon 3 of the β -catenin genes was sequenced to assess the mutation status of all available samples (see Supplementary Information). The biopsy ~~tumour~~ tumor specimens used in this study were obtained with Institutional Review Board approval and clinical information is provided in the Supplementary Information. Biopsies were debrided, dissected into small pieces and frozen in liquid nitrogen. Frozen specimens were immediately placed into TRIzol Reagent (Gibco BRL), homogenized and mRNA isolated as described (Khan *et al.* "DNA Microarray Technology: The Anticipated Impact on the Study of Human Disease" *Biochim. Biophys. Acta* 1423:17-28, 1999; ~~www.nhgri.nih.gov/DIR/microarray~~; each of which is incorporated herein by reference).

Please replace the paragraph beginning on page 31 at line 16, with the following amended paragraph:

The 8,150 human cDNAs used in this study were obtained under a Cooperative Research and Development Agreement with Research Genetics and 6,912 were verified by sequence. This set of cDNAs is part of a larger collection (Khan *et al.* "Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays" *Cancer Res.* 58:5009-5013, 1998; Duggan *et al.* "Expression profiling using cDNA microarrays" *Nature Genet.* 21:10-14, 1999; ~~www.nhgri.nih.gov/DIR/microarray~~; each of which is incorporated herein by reference). On the basis of the Unigene build of 9 March 2000 (~~http://www.ncbi.nlm.nih.gov/UniGene/build.html~~), the 8,150 cDNAs represent 6,971 unique genes in this melanoma array. All clones were confirmed by resequencing if necessary. Microarrays were hybridized, scanned and image analysis performed as described (Khan *et al.* "Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays" *Cancer Res.* 58:5009-5013, 1998; Khan *et al.* "DNA Microarray technology: the anticipated impact on the study of human disease" *Biochim. Biophys. Acta* 1423:17-28, 1999; ~~www.nhgri.nih.gov/DIR/microarray~~; each of which is incorporated herein by reference). The raw data from the microarray is shown in Appendix A, a Microsoft Excel Worksheet, which has been included on a CD-ROM submitted with this application and is incorporated herein by reference.

Please replace the paragraph, beginning on page 36 at line 12, with the following amended paragraph:

Hierarchical clustering of the 31 melanoma samples was performed, resulting in a dendrogram (Fig. 4b 1). Although the dendrogram gives insights about the similarity and relatedness among samples, it does not indicate robustness to variability associated with the assay sampling, etc. In order to draw valid conclusions about the clustering structure present in the data, it is necessary to investigate how variability affects the results of the cluster analysis. To this end, we developed and implemented a method that determines the reproducibility of given levels of cluster structure within the dendrogram under the condition of added noise. The method is described below.

Please replace the paragraph, beginning on page 41 at line 12, with the following amended paragraph:

~~The data used in the survival analysis are shown in Figure 1.~~ A total of 15 cases were included in the analysis, 10 from Group A and 5 from Group B. Survival/follow-up times were rounded to the nearest quarter year. A Kaplan-Meier survival plot was created and log-rank test performed. No statistically significant association between group and survival was found ($p=0.135$).

Please replace the paragraph, beginning on page 51 at line 11, with the following amended paragraph:

Wnt5a scored very high out of all the marker genes analyzed in the ability to discriminate between highly invasive malignant melanoma and less invasive melanoma. Melanoma samples with high levels of *Wnt5a* expression were more aggressive tumors than those with lower levels of *Wnt5a* expression. Figure 6 2B shows the top 22 genes selected for their ability to classify highly invasive malignant melanoma from less invasive melanoma. *Wnt5a* is at the top of the list of these marker genes.

Please replace the paragraph, beginning on page 52 at line 1, with the following amended paragraph:

Figure 6 5 also shows *Wnt5a*'s expected signaling pathway in contrast to the *Wnt1* pathway. *Wnt1* is known to be transforming; however, its proximal methods of signaling are very different from those of *Wnt5a*. In some studies, researchers have observed that the two pathways seem to oppose each other in terms of downstream effects. In the *Wnt5a* pathway, the first transduction of the *Wnt5a* signal is accomplished through the interaction of *Wnt5a* with a G protein-coupled receptor, frizzled 5 (FZD5). The signal is subsequently transduced through the PLC/IP3/DAG/PKC pathways. The *Wnt5a* signal eventually leads to integrin interactions, cytoskeletal effects, and other cellular effects.

Please replace the paragraph, beginning on page 52 at line 9, with the following amended paragraph:

Low level expression of *Wnt5a* in the cluster of 19 melanomas was verified by real time PCR. Data for the samples WM-1791C and UACC-1273 are shown in Figure 7 6A. The real time PCR results show that there is much more *Wnt5a* transcript in cell line WM-1791C, which originally was scored as having high level expression of *Wnt5a* by gene chip analysis, than in UACC-1273, which was originally scored as having low level expression. Vectors used to express higher levels of Wnt5a in cells that normally express low levels were developed using standard techniques to see if the phenotype of less aggressive samples expressing low levels of Wnt5a could be changed. A derivative of UACC-1273, a transfectant 4-3, which had been transfected with this vector, shows an intermediate level of *Wnt5a* expression in the real time PCR analysis. The increase in *Wnt5a* expression carries over in WNT5A protein abundance as shown by Western blot and by immunohistochemical staining (nuclei staining blue, WNT5A staining red) (Figure 7 6B and 6C).

Please replace the paragraph, beginning on page 52 at line 21, with the following amended paragraph:

In terms of morphology, cell lines with originally low levels of *Wnt5a* expression showed dramatic changes in morphology and cytoskeletal organization when stably transfected with a vector driving *Wnt5a* expression. The parental line, UACC-1273, is spindle shaped with few points of attachment to the culture plate and disorganized actin filaments (Figure 8 7). The transfectants are broader and flatter with many extensions and highly polarized actin filaments.

Please replace the paragraph, beginning on page 52 at line 3, with the following amended paragraph:

In order to determine whether there was cross talk between the *Wnt5a* and *Wnt1* pathways, an assay looking at beta-catenin was used. When *Wnt1* signaling is active, beta-catenin is localized to the nucleus. In Figure 9 8A, antibody staining for beta-catenin shows that the beta-catenin is localized in the cytoplasm and not concentrated in the nucleus. Therefore, no cross talk between the two pathways seems to be occurring.

Please replace the paragraph, beginning on page 52 at line 8, with the following amended

paragraph:

Protein kinase C (PKC), a downstream target likely to be modulated by *Wnt5a*, was also looked at. *Wnt5a* modulates PKC activity by phosphorylation of some or all of the PKC isoforms and not by alteration of PKC transcript levels. As can be seen in Figure 8 9, increased phosphorylated PKC is produced in the transfectants expressing significant levels of the *Wnt5a* transcript, as expected. The isoforms most frequently phosphorylated are mu and alpha/beta. This is further evidence that one is looking at the expected *Wnt5a* pathway. PKC is one of the central hubs of signal transduction, and pathways leading to many types of cellular action including proliferation, cytoskeletal organization, and cell movement are known.

Please replace the paragraph, beginning on page 53 at line 17, with the following amended paragraph:

Increased cell movement and invasiveness were also found to correlate with increased *Wnt5a* expression in a scratch assay and a Boyden chamber assay. Transfectants expressing increased levels of *Wnt5a* show increased competence in filling in open gaps on a cell culture dish when compared to cells of the parent cell line (Figure 10 9). Increased phosphorylated PKC was found to correlate with increasing cell invasiveness as measured by a standard test for invasiveness, the Boyden chamber assay.

Please replace the paragraph, beginning on page 53 at line 23, with the following amended paragraph:

The first transduction of the *Wnt5a* signal is accomplished through interaction with a G protein coupled, seven transmembrane receptor, frizzled 5. The various cell lines tested show varying native levels of *fzd5* transcript. In the cell line, UACC-1273, the transition from low to high *Wnt5a* expression is not associated with increasing amounts of the receptor. The use of an antibody to *fzd5* prevents it from responding to *Wnt5a* and thereby attenuates or reverses the phenotypes that increased *Wnt5a* would normally produce. This is shown in the decreased level of phosphorylated PKC upon treatment with the anti-*fzd* antibody and in the decreased invasiveness of *Wnt5a* transfectants treated with the anti-*fzd* antibody (Figure 10).